DESIGN AND DEVELOPMENT OF NANOEMULSIONS AND HYDROGEL BASED SYSTEMS FOR ENHANCED INTESTINAL PERMEABILITY AND CONTROLLED RELEASE OF PHYTOCHEMICALS

SYNOPSIS

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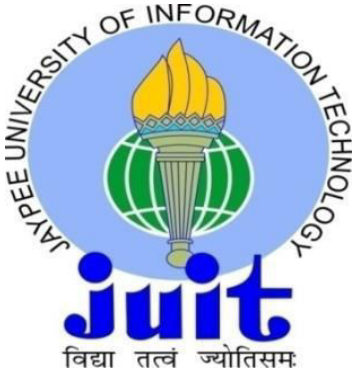

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DESIGN AND DEVELOPMENT OF NANOEMULSIONS AND HYDROGEL BASED SYSTEMS FOR ENHANCED INTESTINAL PERMEABILITY AND CONTROLLED RELEASE OF PHYTOCHEMICALS

ARUN KUMAR

1. INTRODUCTION

An ideal drug delivery system fulfils the objective of maximizing therapeutic effect while minimizing toxicity. With the progress in time and advances in science and technology, dosage forms have evolved from simple mixtures and pills, to highly sophisticated systems, which are known as novel drug delivery systems. Nanoemulsions are novel drug delivery systems consisting of emulsified oil and water systems with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm and can exist as oil-in-water (o/w) or water-in-oil (w/o) form, where the core of the particle is either oil or water, respectively. Nanoemulsions are made from pharmaceutical surfactants that are generally regarded as safe (GRAS). The capacity of nanoemulsion to dissolve large quantities of low soluble drugs along with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation make them ideal drug delivery vectors [1]. The major advantages of nanoemulsion as drug delivery carriers include increased drug loading, enhanced drug solubility and bioavailability, reduced patient variability, controlled drug release, and protection from enzymatic degradation [2].

A lot of techniques are available for enhancing absorption of poorly water-soluble drugs, like use of lipid-based systems. Thus enhancement of aqueous solubility in such case is a valuable goal to successfully formulate them into bioavailable dosage forms. A range of novel strategies are currently being developed for efficient delivery of poorly water-soluble drugs, such as the formulation of amorphous solid form, nanoparticles,

microemulsion, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes. Among all, the most accepted approach is the lipid-based formulation approach [3, 4]. Lipid-based formulations enhance the absorption by enhancing solubilization, prolonging gastric residence time, stimulating the intestinal lymphatic transport pathway, altering intestinal permeability, reduced activity of efflux transporters and reduced metabolism.

Nanoemulsions and self nanoemulsifying drug delivery system (SNEDDS)

Nanoemulsions are a colloidal particulate system in the submicron size range acting as carriers of drug molecules. Their size varies from 10 to 1,000 nm. These carriers are solid spheres and their surface is amorphous and lipophilic with a negative charge. As a drug delivery system they enhance the therapeutic efficacy of the drug and minimize adverse effect and toxic reactions. An emulsion is a biphasic system in which one phase is intimately dispersed in the other phase in the form of minute droplets ranging in diameter from 0.1 to 100 μm. It is a thermodynamically unstable system, which can be stabilized by the presence of an emulsifying agent (emulgent or emulsifier). The dispersed phase is also known as internal phase or the discontinuous phase while the outer phase is called dispersion medium, external phase or continuous phase. The term 'nanoemulsion' also refers to a miniemulsion which is fine oil/water or water/oil dispersion stabilized by an interfacial film of surfactant molecule having droplet size range 20–600 nm. Because of small size, nanoemulsions are transparent.

Self-nano emulsifying drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that form fine oil-in-water nanoemulsion when introduced into aqueous phases under gentle agitation. SNEDDS spread readily in the gastrointestinal tract, and the digestive motility of the stomach and the intestine provide

the agitation necessary for self-emulsification [5]. SEDDSs typically produce emulsions with turbid appearance, and droplet size between 200 nm to 5 μm, while self micro emulsifying drug delivery systems (SMEDDSs) form translucent micro-emulsions with droplet size of less than 200 nm. However, self nano-emulsifying drug delivery systems (SNEDDS) produce clear or transparent emulsion with droplets size less than 100 nm [6, 7]. The factors influencing the phenomenon of self nano-emulsification are:

- The physicochemical nature and concentration of oily phase, surfactant and co-emulsifier or co surfactant or solubilizer (if included).
- The ratio of the components, especially oil-surfactant ratio.
- The temperature and pH of the aqueous phase where nano-emulsification would occur.

Research gap related to low intestinal permeability of herbal products

Herbal products are now a day's one of the most utilized products and effective drug guide for new chemical entities and discovery [8]. Recently, many research based pharmaceutical companies and even WHO encourages use of medicinal herbs of natural origin because chronic disease requires long term therapy, and using synthetic drugs in that case may cause dubious adverse effects [9]. Herbal extracts are now a day's one of the most widely used medicine due to their ease of availability and low production cost. During the period of 2001-2010, 34 herbal based drugs were approved by Food and Drug Administration (FDA) and around 100 new products are under clinical trials, showing the importance of herbal products in drug development [10].

Intestinal absorption or permeation is a key factor for the bioavailability of oral dosage forms. It is well-known that influx and efflux transporters such as P-glycoprotein (P-gp) expressed on the membrane of epithelial intestinal cells have a substantial impact on drug

absorption. Influx transporters facilitate drug absorption, whereas efflux transporters prevent the drug absorption. P-gp alters intestinal permeation of hydrophobic compounds by averting the influx into cells and facilitates drug efflux from intestinal cells back into the lumen. Inhibiting P-gp may improve drug absorption across intestinal barriers [11, 12]. P-gp inhibitors act as high avidity substrates (e.g. verapamil, quinidine, Tween 20, 80, Span 20) or block its function by binding to it (e.g. sulfhydryl-substituted purine) [13].

Strategies for improving the intestinal permeability of herbal products

In order to improve the intestinal permeability of herbal products novel lipid based drug delivery system has been incorporated along with P-gp inhibitors (Tween 20, 80, span 20 and PEG 400) as pharmaceutical excipients. The design of lipid based formulations includes two technologies

1. Designing of novel drug delivery system that will bypass efflux mechanism and hepatic metabolism.
2. Administration of P-gp inhibitors (Tween 20, 80, Span 20 and PEG 400 as surfactant)

These two technologies are easily feasible due to advances in novel lipid and oil based approach. Fortunately, certain surfactants used in lipid based formulation have the ability to inhibit P-gp mediated efflux mechanism and P-gp inhibitors [14, 15]. Therefore co-administration of herbal products with suitable pharmaceutical excipients (P-gp inhibitors) in optimized concentration can be used to improve the intestinal permeability, oral bioavailability and the targeting of drug to a particular site.

Hydrogels and silver nanoparticles

The term hydrogel is used to describe materials that are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water. Hydrogels have

important applications in the areas of controlled drug delivery, as coatings in pharmaceutical applications and as dissolution and binding agents in tablets. In general, hydrogels are of two type semi-IPN (interpolymer network), in which one polymer is cross-linked and other is free while full IPN involves the cross-linking of both the hydrogel network.

The use of silver based antimicrobial agents has emerged due to the fact that an increase of bacterial resistance to antibiotics caused by their wide spread use. Although these topical agents exhibit superior bacterial inhibitions, there is a delay in the wound healing process. To solve this particular problem, silver is used in the form of colloidal suspension, i.e., silver nanoparticles (AgNPs). An improved bactericidal activity is attributed to AgNPs because of their electronic effects that results a change in the local electronic structure of the surfaces of the nanosized particles, i.e., enhancement of the reactivity of AgNPs surfaces. The AgNPs are capable of inactivating the vital enzymes and also help in prevention of the replication of DNA.

Research gap related to silver nanoparticles

The synthesis technique, instability, immediate release and local toxicity of silver are major concern for its biological application as reduction of silver salt using synthetic chemicals such as sodium borohydride is hazardous to environment while high concentration of silver in wound tissue adversely affects nascent tissues and thus hamper the healing progression [16].

Strategies to overcome the above problems

To improve stability, controlled release, toxicity issue, PVA/Chitosan and sprayed hydrogel dressings (SHD) loaded with silver nanoparticles were synthesized using green chemistry (phytochemicals as reducing agent) to tackle the issues related with the toxicity and immediate release of silver. AgNPs exert its effect by lowering the bacterial burden

in the ulcers, while phytochemicals does it by reducing the oxidative stress. The combination of AgNPs and hydrogel dressing further overcome the problems related with mechanical property of hydrogels and silver toxicity as it releases in a controlled manner from the polymer matrix system.

Hydrogels offer large free spaces between the cross-linked networks in the swollen stage that can act as a nanoreactor for the nucleation and growth of the nano-Ag. This approach offers short reaction times, the use of phytochemicals as reducing agent, high efficiency in converting the silver cations (Ag^{+}) to nano-Ag and control over the size of nano-Ag has been welcomed for the delivery of silver ions as an antimicrobial agent.

1. OBJECTIVES OF THE STUDY

In order to improve the controlled release and intestinal permeability the objectives of our study has been categorized in to two parts:

Objective 1: **Design and development of lipid based nanoemulsions for enhanced intestinal permeability**

Phase 1. Design and development of Tinospora Cordifolia extract loaded nanoemulsion for improved intestinal permeability.

Phase 2. Design and development of Berberis aristata extract loaded SNEDDS for controlled release and improved permeation.

Objective 2: Design and development of silver nanoparticles and phytochemicals loaded polymeric hydrogel for controlled release.

Phase 1. Formulation and evaluation of silver nanoparticles and Ocimum sanctum extract loaded PVA/ chitosan hydrogels for controlled release

Phase 2. Design and synthesis of silver nanoparticles and Ficus benghalensis extract loaded nanocomposite sprayed hydrogel dressings.

Objective 1

Phase 1. Design and development of *Tinospora Cordifolia (TC)* extract loaded nanoemulsion for improved intestinal permeability.

Clinical efficacy of phytotherapeutics has been limited due to their poor stability, solubility and oral bioavailability [17]. In this study, TC extract loaded nanoemulsion are optimized using clove oil as lipid phase while Span 80 and Tween 20 were used as lipophilic surfactant and hydrophilic surfactant in order to enhance drug loading and permeation across the intestinal membrane. The prepared nanoemulsion are evaluated for particle size, zeta potential, drug release, stability study and *in-vitro* permeation study using non-everted gut sac method. The mean particle size of formulated nanoemulsion was found within the range of 33.7 nm to 377 nm. It has been found that the active components remained stable in the optimized nanoemulsion during study for 3 months which further releases 79.4±1.5% and 80.5±0.9% of extract from solution and nanoemulsion after 12 h and 20 h, respectively. Approximately 3.95 fold enhancement in permeability coefficient of TC extract was noticed upon formulating it as nanoemulsion in comparison to control by everted gut model.

METHODS

- Nanoemulsions (NE) were prepared by solvent evaporation method.
- Construction of pseudo-ternary phase diagram
- *In-vitro* characterization involved stability study, drug loading and drug release study.
- *In-vitro* drug permeation study using non-everted gut sac method.

RESULTS AND DISCUSSION

In this study, nanotechnological based approach has been applied towards enhancement in the oral bioavailability and intestinal permeability of TC by inhibition of p-glycoprotein efflux pump along with improvement in stability of phytochemicals in GIT.

The particle size distribution showed that on increasing the concentration of span 80, droplet size of NE increases while, the reverse effect was observed by increasing the co-surfactant concentration. Based on the droplet size and zeta potential an optimized formulation consisting of clove oil, Span 80, Tween 20 and ethanol (7.7:4:0.5:21 w/w) and loaded with 83.8% of TC stem extract. The mean particle size of formulated nanoemulsion was found within the range of 33.7 nm to 377 nm (Figure 4).

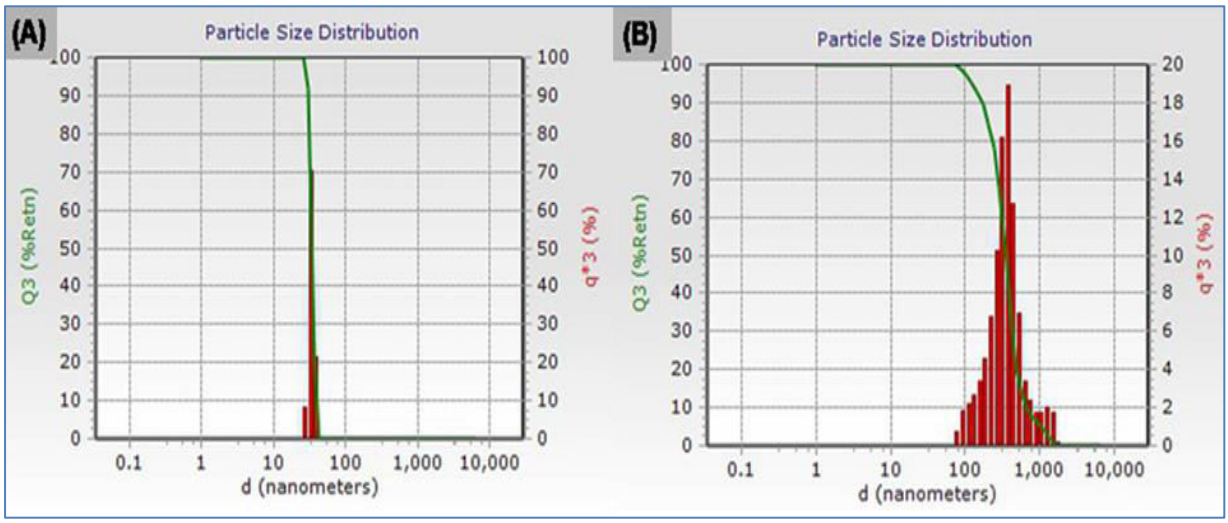


Figure 4. Particle size distributions of (A) blank nanoemulsion and (B) nanoemulsion containing *Tinospora cordifolia* extract (F5).

From the release profile study it was observed that 79.4±1.5% and 80.5±0.9% of extract were released from solution and nanoemulsion after 12 h and 20 h, respectively (Figure 5). Approximately 3.95 fold enhancement in permeability coefficient of TC extract was noticed upon formulating it as nanoemulsion in comparison to control by everted gut model.

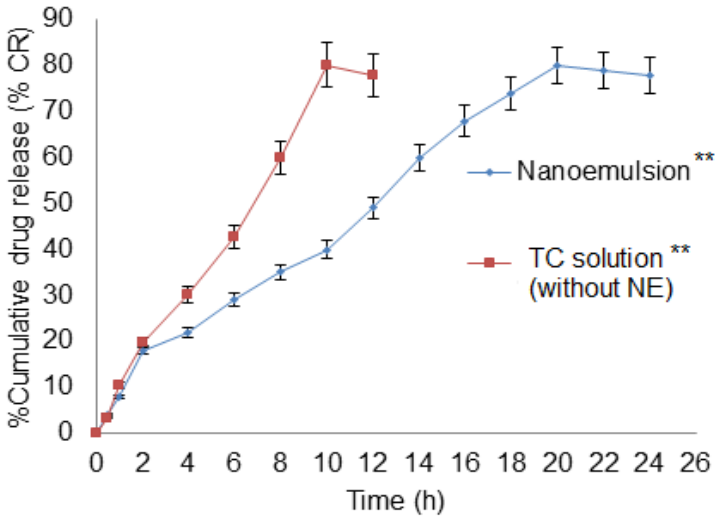


Figure 5. Drug release profile study of nanoemulsion and TC solution

Phase 2. Design and development of *Berberis aristata* (BA) extract loaded SNEDDS for controlled release and improved permeation.

In this study, we design and develop self nanoemulsifying drug delivery system (SNEDDS) containing *Berberis aristata* (BA) extract with an objective of improving its oral anti-diabetic efficacy as well as permeation across the biological membrane. The prepared SNEDDS were further evaluated for particle size, zeta potential, particle size distribution using fluorescent microscopy, drug release study and *in-vitro* permeation study using non-everted gut sac method. Nano-droplet size and zeta potential of nanoformulations were optimized using two surfactants, i.e. Tween 80 and Pluronic F 68, noted as 145.7 nm, 147.1 nm and -23.30 mV, -31.2 mV, respectively. Sustained release profile of the extract from SNEDDS was examined using UV-visible spectrophotometer (λ_{max}; 350 nm), was found up to 34 h which further showed greater reduction of glucose diffusion i.e. ~126% of raw extract and ~138% of control for 120 mins. Moreover, approximately 5.77 and 5.86 fold enhancement of *ex-vivo* permeability coefficient of *BA* was noticed upon formulating it as SNEDDS using two surfactants i.e. Tween 80, Pluronic F68, respectively.

METHODS

- SNEDDS were synthesized using self-emulsification method.

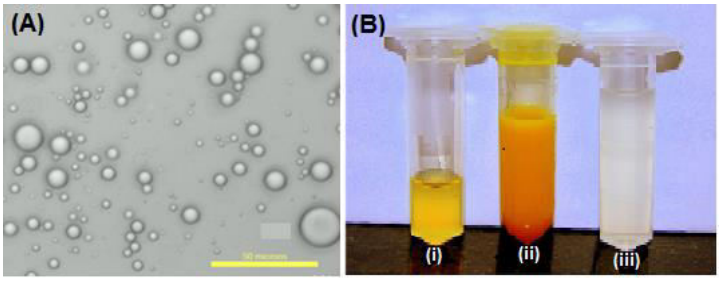


Figure 6. (A) Phase contrast image of SNEDDS (B) showed (i) blank SNEDDS, (ii) BA loaded SNEDDS and (iii) diluted SNEDDS with distilled water

- *In-vitro* characterization involves estimation of particle size and zeta potential, drug loading, morphological study and drug release study
- Non-everted gut sac method for intestinal permeation

RESULTS AND DISCUSSION

This research work is focused on utility of SNEDDS towards enhancement in the oral bioavailability, intestinal permeability and *in-vitro* antidiabetic activity with minimization of stability issue related with phytochemicals like degradation of extract in the acidic pH of the stomach by absorption through lymphatic system. The smaller size (~ 144 nm) and high zeta potential (- 43.7 mV) of these nanoemulsion showed a controlled release across the dialysis membrane (> 90 %) for up to 34 h. It has been found that on increasing the concentration of tween 80 from 10 to 50%, decrease in average droplet size was observed such a decrease in the droplet size may be due to the formation of rigid interface between the oil and water, which provides stable surface by reducing the interfacial tension.

Furthermore, to investigate the utility of the prepared SNEDDS formulation as self emulsification, 0.1 ml of SNEDDS was mixed with 100 ml of distilled water in a glass beaker at room temperature. The tendency to emulsify spontaneously within the distilled water was assessed visually using the grading criteria according to Shafiq et al., 2007. Rapidly forming formulations with a clear microemulsion (with in 1 min; labeled as A). If bright white emulsion is formed (with in 2 min; labeled as B) was still considered to have meet the self-emulsification criteria. A dull, grayish white emulsion with either poor or less emulsification region with large oil drops floating on the surface was labeled as "C" in the ternary phase diagram (Figure 7).

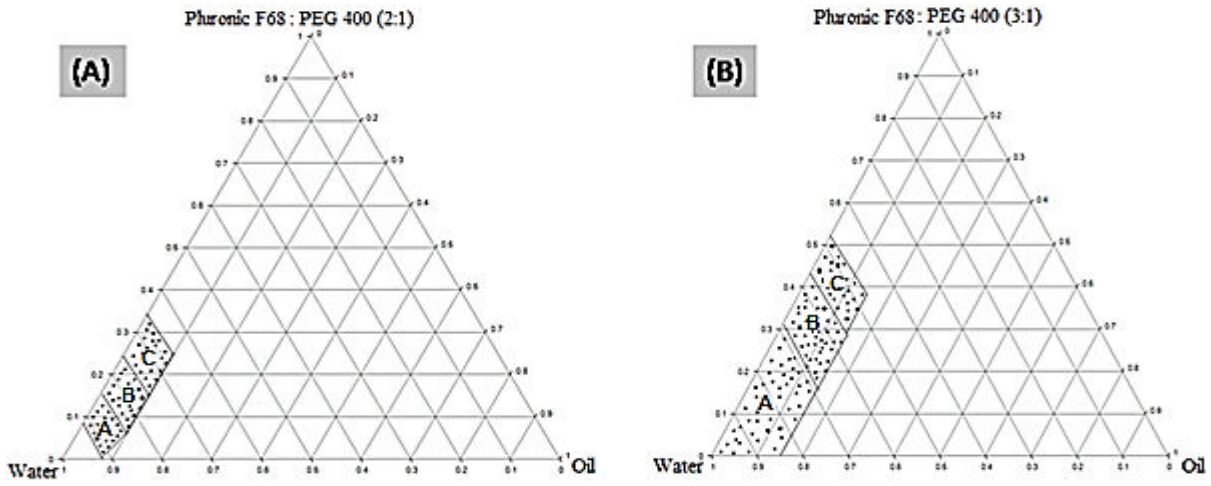


Figure 7. Pseudo-ternary phase diagram showing self nanoemulsifying region with varying concentration of Pluronic F68 with respect to PEG 400.

Moreover, Controlled release of BA (22 h) results in the inhibition of free radicals which further confirms that sufficient polyphenolic compounds were released over a period of 22 h. Approximately 5.86 fold enhancements in the intestinal permeability was observed on formulating it as SNEDDS to provide a tangible link between the absorption and permeation of the drug with its bioavailability (Figure 8).

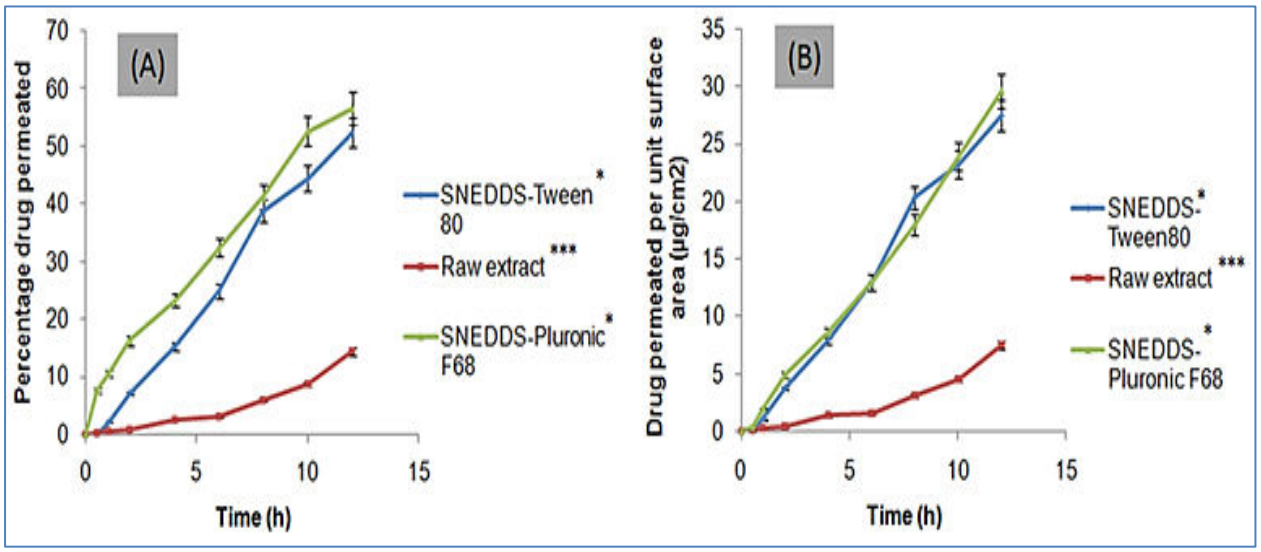


Figure 8. (A) Showed percentage drug permeated (%), (B) drug permeated per unit surface area (µg/cm^2).

CONCLUSION

In our study NE approach has been successfully explored for controlled delivery and improved permeation of TC extract and BA extract form nanoemulsion and SNEDDS, respectively. The droplet size below 100 nm (~33.7 nm) and controlled release of80.5±0.9 and ~ 95% of TC extract and BA extract, respectively are the unique features of these nanoemulsions. The prepared nanoemulsion has low loading efficiency as some of the drug diffused out during sonication of the nanoemulsion essential for low particles size. To ensure high drug loading, SNEDDS of BA are prepared using self emulsification technique and is a promising strategy for improved drug loading, stability, permeation across the intestinal membrane and in turn, will enhance the therapeutic efficacy and bioavailability of BA with varying surfactant and oil types to achieve maximum drug loading, sustained drug release profile and size range, in tunable manner. The developed novel formulation results in 5.86 fold enhancement in the permeability parameters. Overall, these studies demonstrated production of stable SNEDDS and a sustained drug release profile for up to 32 h.

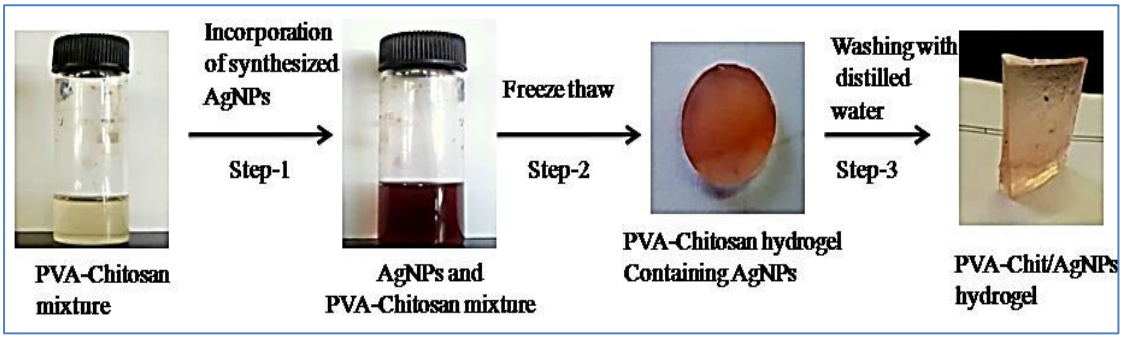
Objective 2

Phase 1. Formulation and evaluation of silver nanoparticles and *Ocimum sanctum* loaded polyvinyl alcohol (PVA)/Chitosan hydrogels for controlled release

In the recent years, potential application of phytochemicals for the synthesis of silver nanoparticles is a significant approach towards the field and application of antimicrobial nanomaterials for wound healing applications [18]. In this study, research efforts have been applied to synthesize PVA/chitosan-silver based hydrogels using freeze thaw process so as to bypass the toxic effects associated with chemical cross-linking, where both phytochemicals and silver nanoparticles could retain their individual effects and characteristics in the system with improved mechanical and stability properties. The prepared hydrogels were further evaluated for swelling index; thermal degradation, surface morphology and compatibility studied of hydrogels were confirmed by TGA, SEM and FTIR studies. A controlled release of 84.3 % (28 h) of *Ocimum sanctum* extract was noticed from hydrogel discs which scavenges 69.2 % of free radicals as compared to raw extract 82.5 % (16 h) which scavenges 63.1% of free radicals.

METHODS

- Synthesis of PVA/CH hydrogel using freeze thaw method.



- *In-vitro* characterization: Swelling, degradation, mechanical testing of prepared hydrogels, morphological study using SEM, polymeric interaction through FTIR and degradation profile through TGA etc.

RESULTS AND DISCUSSION

This unique synthesis of PVA/CH/AgNPs hydrogels route was based on physical blending of different proportions of water soluble PVA and chitosan solution. It has been found that size of AgNPs increases as we increase the amount of OS extract while opposite results were obtained in case of zeta potential i.e. zeta potential of AgNPs decreases from -7.7 to 10.6 mv. To determine whether the prepared AgNPs are bacteriostatic or bactericidal, the countable colony of bacteria was achieved by sequential dilution. The MIC of AgNPs against *S. aureus* and *E. coli* was observed as 42 µg/ml and 72 µg/ml.

The polymeric interaction through FTIR showed a relative reduction in the intensity of the peak at 1637 cm^{-1} has been noted due to deformation vibration of the amine group of chitosan (Figure 9). All other peaks of chitosan and PVA maintained their position in the corresponding PVA/CH and PVA/CH/Ag hydrogels with a little change in their peak intensity.

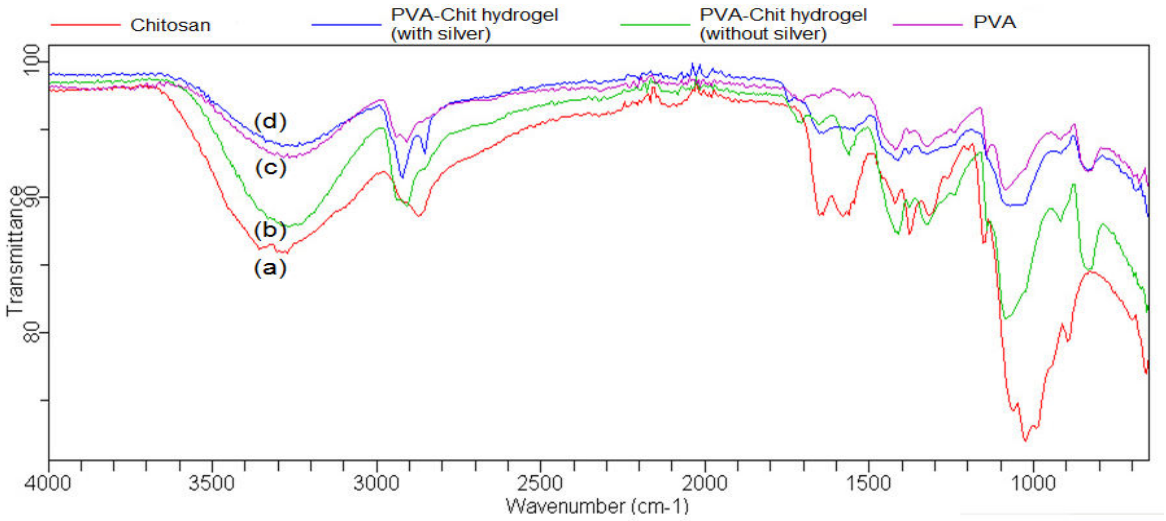


Figure 9. Demonstration of comparative ATR-FTIR spectra of (a) chitosan, (b) PVA/CH hydrogel, (c) PVA and (d) PVA/CH hydrogels (with AgNPs)

To evaluate the degradation behavior Thermo gravimetric analysis (TGA) was performed with chitosan, PVA, PVA/CH and PVA/CH/Ag hydrogels followed by thermal degradation of hydrogels at a heating rate of 10 ºC/min. Blank PVA/CH hydrogels and PVA/CH/Ag hydrogels degrade in two steps and accomplished by ~96 % and ~ 94% weight loss. To evaluate the strength and elasticity of prepared hydrogel mechanical testing was performed with increase PVA and AgNPs concentration. It has been found that force and extension of prepared hydrogel increases as we increase the concentration of PVA from 33 to 100%. Moreover, in case of PVA/CH hydrogels containing silver nanoparticles force and extension were also increased from 0.49 to 1115 N and 45 to 129 mm, respectively (Figure 10).

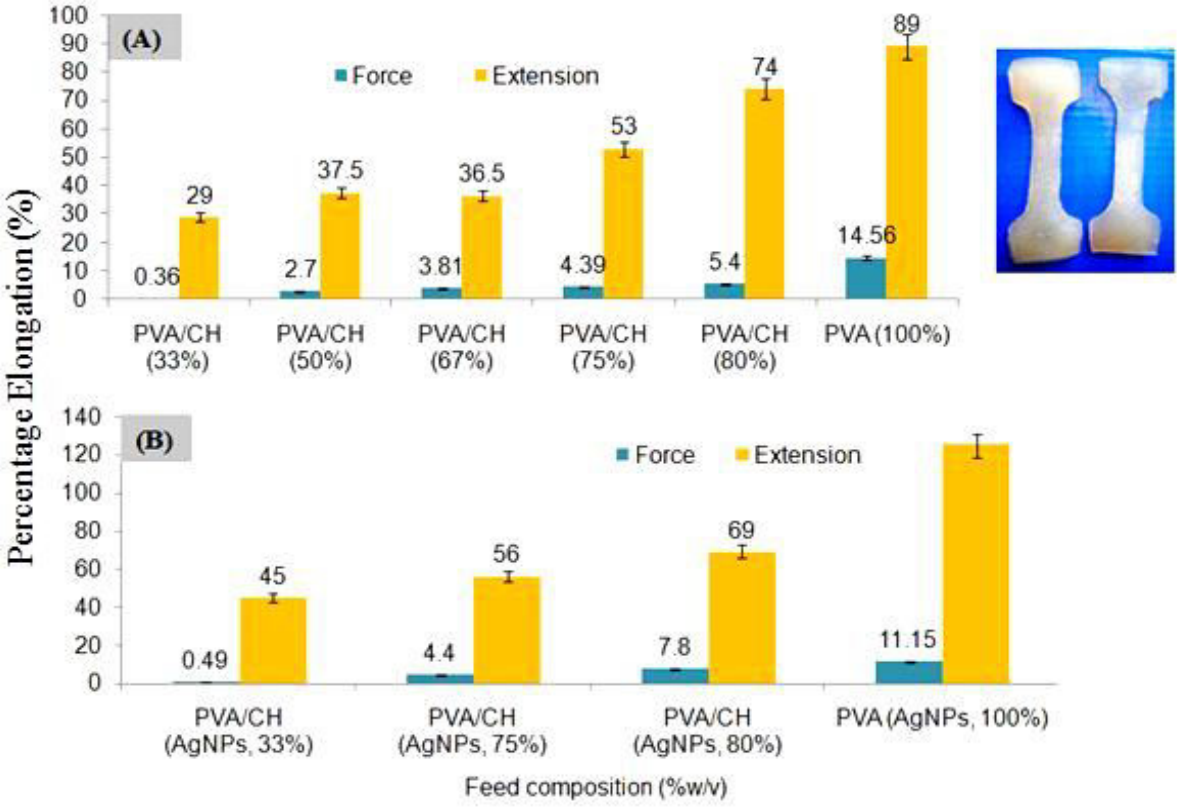


Figure 10. Graph showing force and extension values (A) PVA/CH hydrogel without silver nanoparticles, (B) PVA/CH hydrogels loaded with silver nanoparticles.

The effect of chitosan content on the ESR with respect to different pH media was also investigated. It has been found that ESR increased from 0.96 to 6.89 (7.1-fold, at pH 1.2) on increasing the content of CH (0% to 100%), while ESR increased from 2.9 to 5.21 (1.79-fold; pH 6.5) on increasing the PVA content (50% to 100%) in the PVA/CH hydrogels (Figure 11). SEM images demonstrate that homogeneity of the prepared hydrogels was mostly due to the interaction of hydrogen bonds among the functional group of the blend component (Figure 12). The Hydrogels containing AgNPs and AgNPs solution releases ~84 % and 83.9% of Ag ions in 30 and 20 h respectively. The drug release kinetics was analyzed by plotting the log of cumulative release data versus log of time. According to Korsmeyer-Peppas model, the values of PVA/CH/AgNPs and PVA/CH/OS hydrogels exponent 'n' is found to be >1 which indicated a super case-II transport.

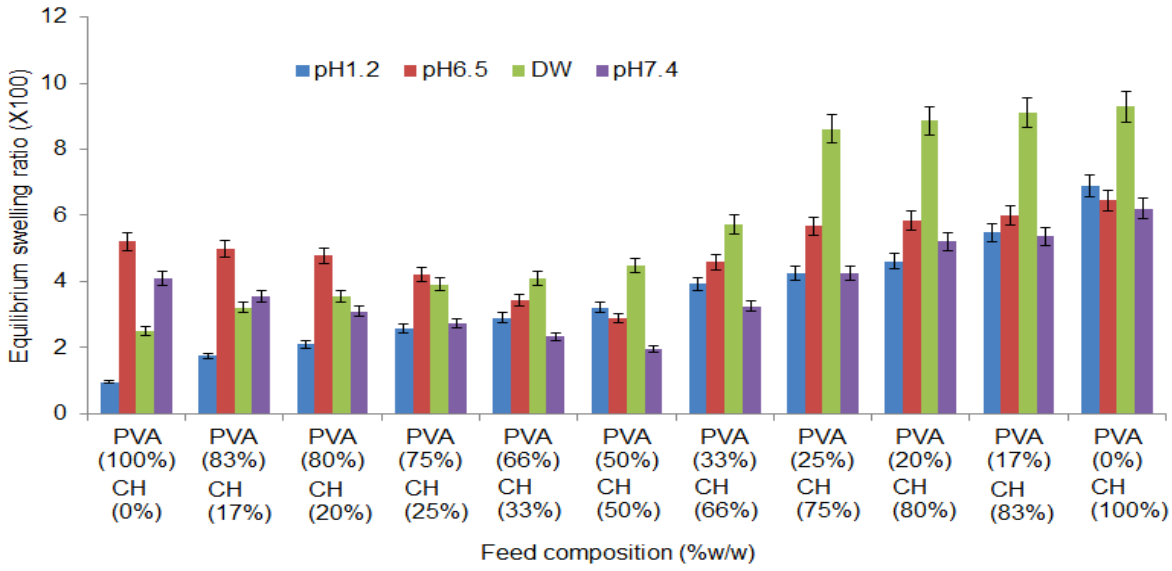


Figure 11. Demonstration of effect of PVA/CH ratio on the water uptake behavior (equilibrium swelling ratio; ESR) of PVA/CH hydrogel in distilled water (DW), pH 1.2, 6.5 and 7.4 (n=5; mean ± SD)

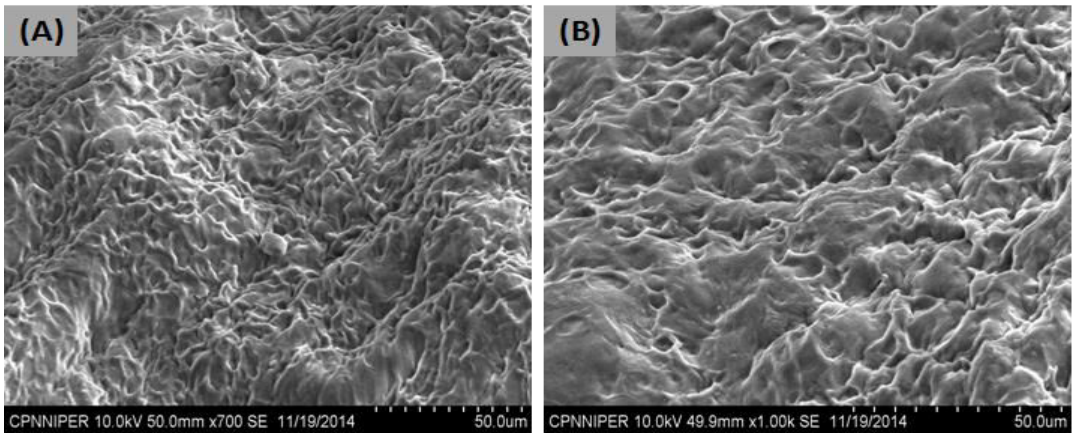


Figure 12. SEM images demonstrated roughness and surface morphology of PVA/SA SHD films (1:1) (at magnification of 100x and 500x (Inset), respectively.

Phase 2. Design and synthesis of silver nanoparticles and *Ficus benghalensis* (FB) extract loaded nanocomposite sprayed hydrogel dressings

An innovative concept of spontaneous film dressing has been designed using polyvinyl alcohol (PVA) and sodium alginate (SA) as synthetic and natural polymeric components and, cross-linked with boric acid and calcium chloride respectively. Silver nanoparticles (AgNPs) are synthesized by green chemistry using *Ficus benghalensis* extract (FB). The prepared hydrogels were further characterized for swelling, degradation, FTIR, SEM, TGA, antioxidant, drug release and antimicrobial activity. FTIR spectra showed polymeric interaction with AgNPs while SEM images show outer surface of the SHD film. Equilibrium swelling and degradation in aqueous media (distilled water and buffers) are found to dependent upon PVA/SA ratio. Uniformly distributed AgNPs (particle size ~27.55±2.01 nm), high water retention (~13 fold) and biodegradable (~5 days) nature of dressing showed a sustained release profile of AgNPs for up to 24 h with concentration-dependent antimicrobial activity.

METHODS

- Synthesis of AgNPs loaded PVA/SA SHD using cross-linking method.
- *In-vitro* characterization: swelling index, degradation behavior, morphological study using SEM, thermal degradation using TGA, polymeric interaction using FTIR, drug release study and antimicrobial assay for zone of inhibition.

RESULTS ANDDISCUSSION

In this study sprayed hydrogel dressings loaded with silver nanoparticles has been explored as a novel approach for wound management as it offers proper adherence to the wound site contamination free, self-shaped protective covering without wrinkling or

fluting in the wound bed with excellent patient compliance. The particle size of prepared AgNPs was found in the range of 27.55±2.01 nm with pdi of 0.66. Figure 13 (A&B) showed aerosol system used for spraying mixture of PVA/SA and boric acid/calcium chloride as cross-linkers, while figure 7B showed strength, flexibility and elasticity of prepared film.

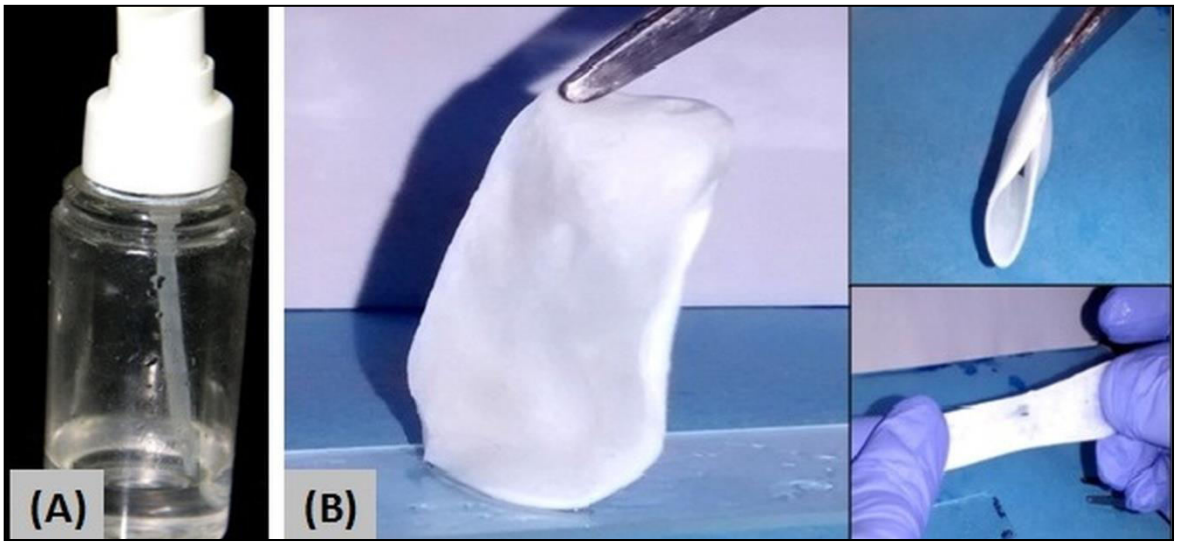


Figure 13. Photograph of SHD films demonstrated (A) sprayers used for spraying mixture of PVA/SA and BA/$CaCl_2$, (B) strength of SHD film (inset, image showed flexibility and elasticity of SHD films).

Silver ions (Ag^+) is an antimicrobial agent in nature while it is unstable to store as such, therefore, Ag NPs (Ag^+) have been designed to store as reservoir of Ag^+ which on release in the medium, act as antimicrobial agent. Minimum inhibitory concentration (MIC) of AgNPs against *S. aureus* and *E. coli* was observed as 47 µg/ml and 61 µg/ml of AgNPs (Figure 14 (A, B, C and D). The variation in the MIC of gram positive and gram negative bacteria to silver nanoparticles might be due to the difference in the thickness, susceptibility and constituents of their membrane structure.

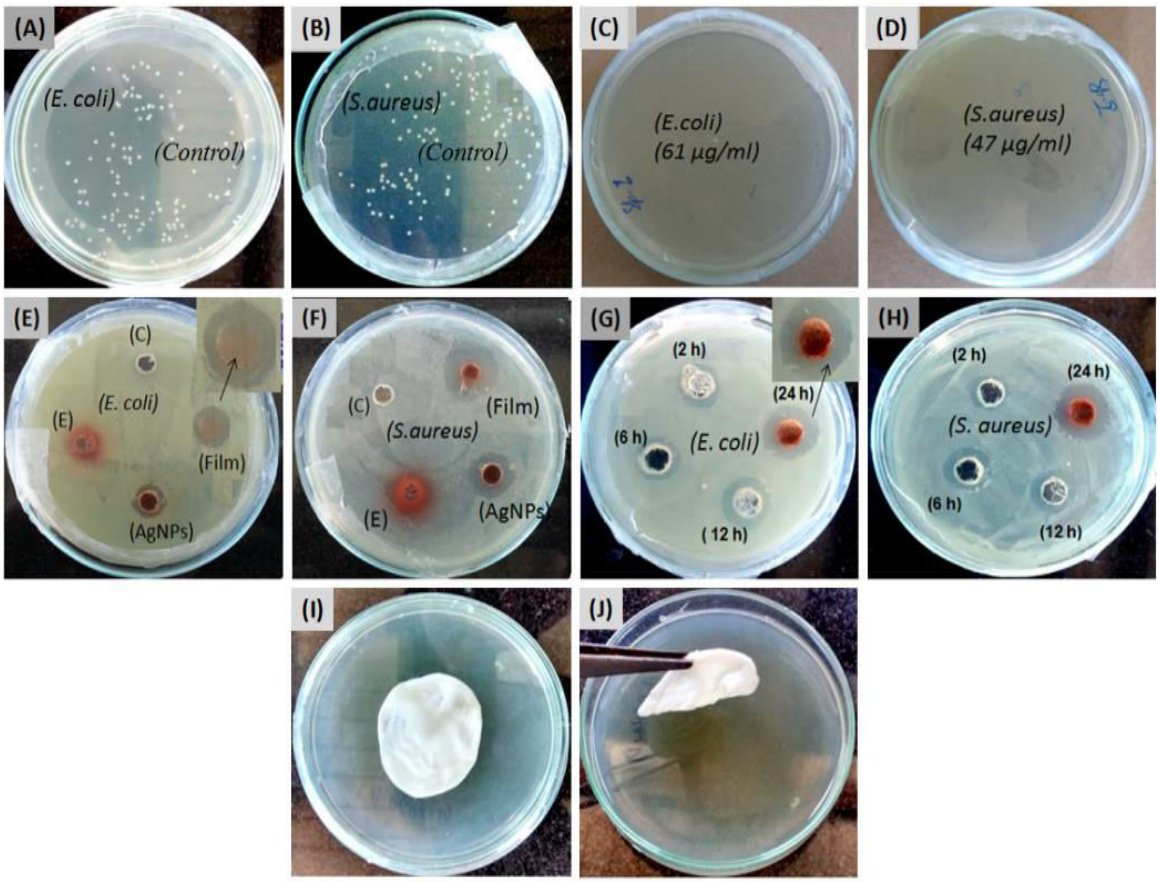


Figure 14. Photographs (A, B) showing MIC of control (C & D) MIC of AgNPs against *E. coli* and *S. aureus*, figure (E&F) showing zone of inhibition (ZOI) as control (C), FB extract (E), AgNPs and PVA/SA/Ag SHD films against *E. coli* and *S. aureus,* (G&H) showed the ZOI by Ag ions released from sprayed hydrogel after 2, 6, 12 and 24 h, respectively, while (I&J) showed impermeability of bacteria across the SHD films.

The polymeric interaction from the FTIR spectra showed that the –COO– band of silver film has shifted to higher wave number i.e. from 1614 cm^{-1} to 1670 cm^{-1} indicated a strong interaction between SA, PVA and AgNPs. This shifting can be attributed to the formation of coordination bond between the electron rich groups (such as C=O and OH) present in the SHD hydrogel network (Figure 15).

The surface morphology of dried films showed a wrinkled, non-porous surface with uniformity which may have participated in sustained release of the *FB* extract and Ag nanoparticles (Figure 16).

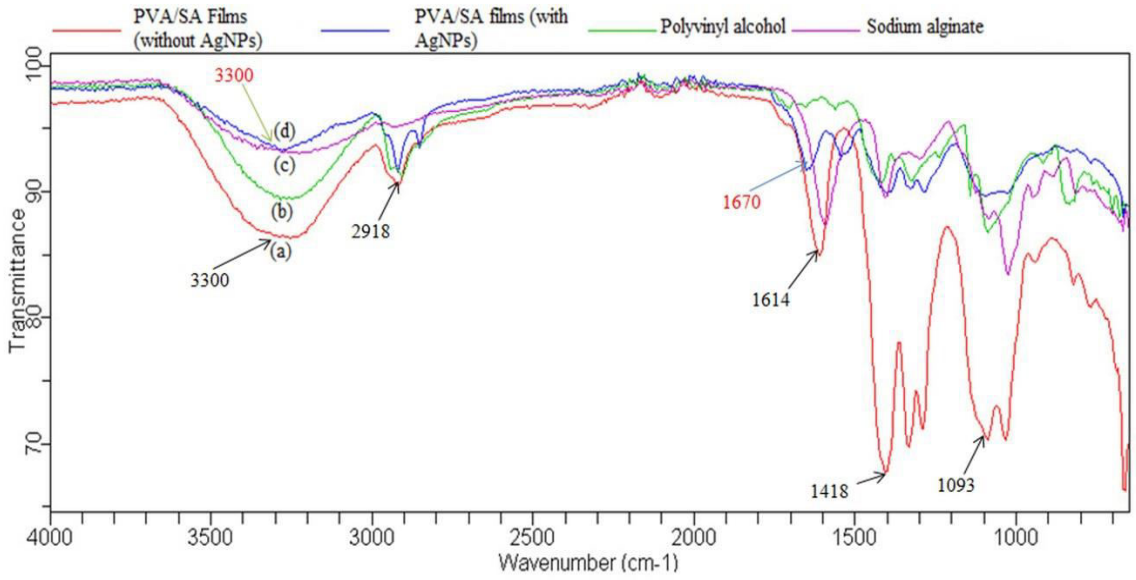


Figure 15. Demonstration of comparative ATR-FTIR spectra of (a) PVA/SA SHD films(without AgNPs), (b) PVA, (c) Sodium alginate (SA) and (d) PVA/SA film with AgNPs.

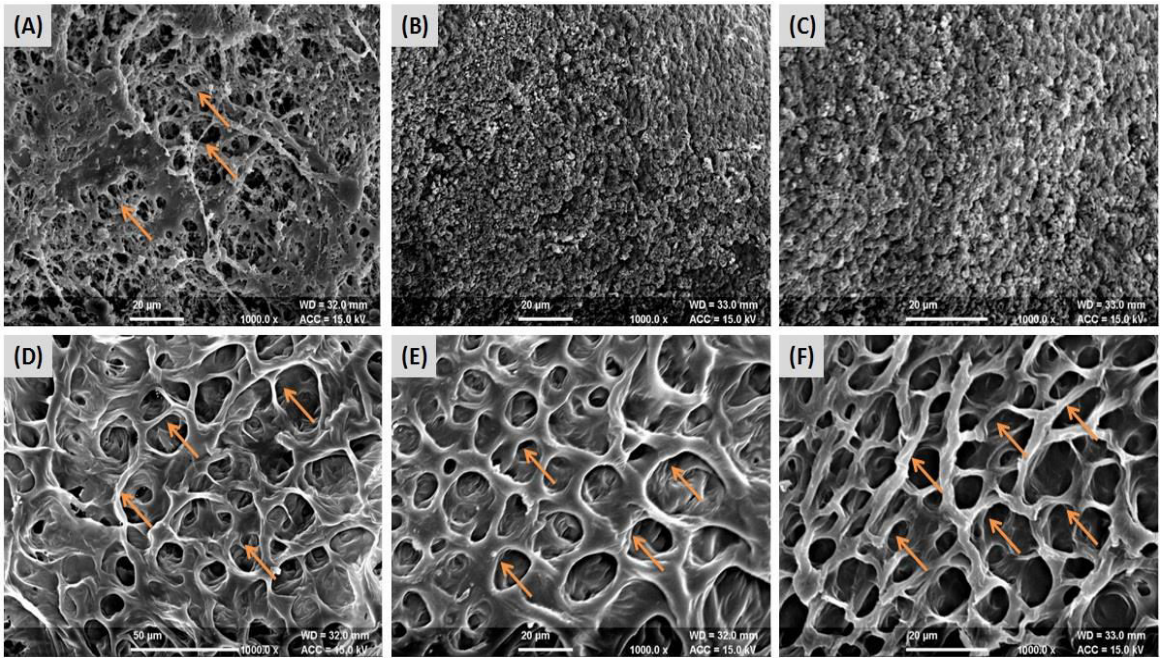


Figure 16. SEM images (A)-(F) depicts the surface morphology, porosity and cross-linking of PVA/SA SHD films with PVA/SA ratio varying in the range of 1:0, 0:1, 1:2, 1:1, 2:1 and 3:1(at magnification of 1000x).

Finally, to investigate the effect of AgNPs on polymeric thermal stability, thermo-gravimetric analysis was performed with PVA, SA alone, blank SHD films and SHD film

loaded with AgNPs at a heating rate of 10 ℃/min. Blank SHD films and SHD films loaded with AgNPs degrade in two steps and accomplished by ~86 % and ~ 65% weight loss. The difference in the decomposition between blank SHD and SHD film loaded with AgNPs was found ~21%. The presence of nano-silver in the hydrogel network can catalyze CO_2 elimination from polymer chain and thus delayed degradation process (Figure 17).

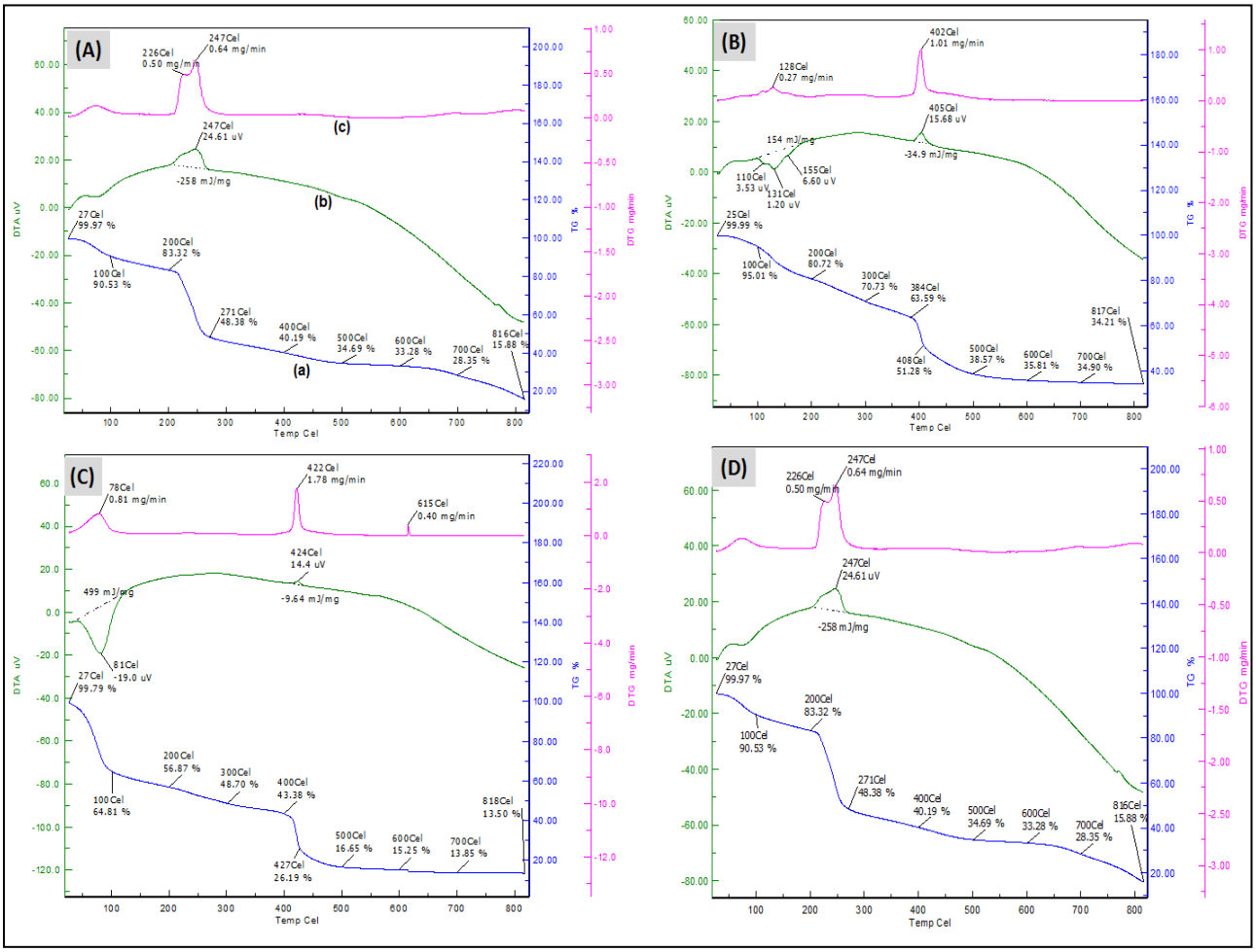


Figure 17. TGA studies showed polymeric interaction through thermal degradation behavior of PVA, SA, PVA/SA SHD and PVA/SA/Ag SHD hydrogels at a heating rate of 10 ℃/min.

It has been found that equilibrium swelling ratio (ESR) decreased from 6.89 to 4.27 (1.61-fold; at pH 1.2) on decreasing the content of SA (100% to 66%) while ESR

increased from 0.33 to 3.39 (10.27-folds; pH 1.2) on increasing PVA content (33% to 100%) in the PVA/SA SHD (Figure 18).

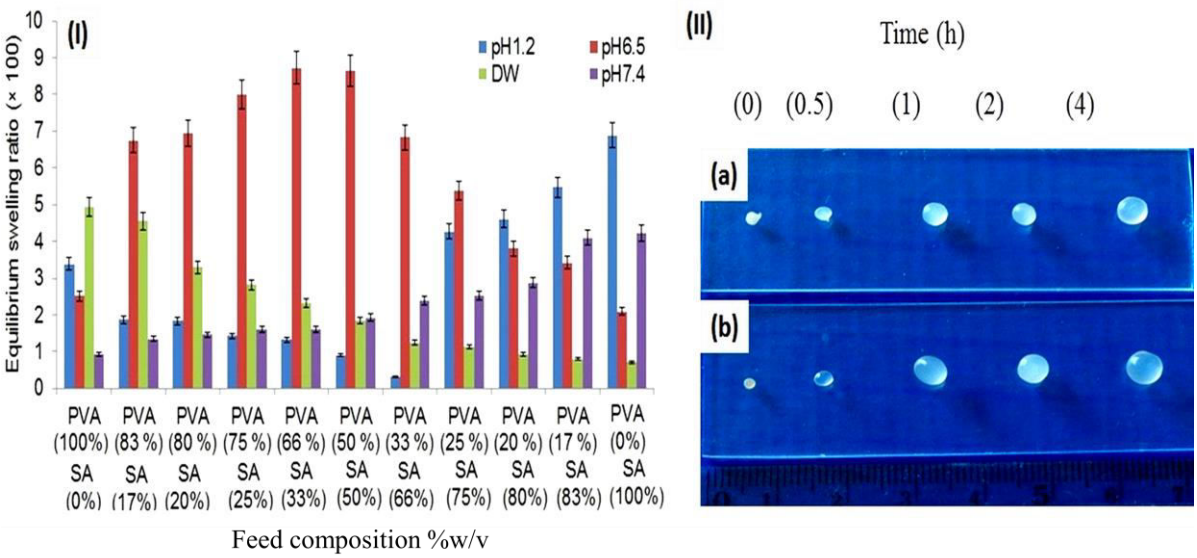


Figure 18. (I) Demonstration of the effect of PVA/SA ratio on the water uptake behavior (ESR) in different pH (n= 5; mean ± SD), (II) Photographs of (a) SA and (b) PVA beads showed degree of swelling after 30 min, 1, 2 and 4 h, respectively.

It was noticed that ~87 % and ~82 % of *FB* extract and silver ions were released in a sustained manner up to 24 h, respectively. According to Korsmeyer-Peppas model, the values of SHD/AgNPs and SHD/*FB* films exponent 'n' is found to be >1 which indicated a super case-II transport.

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Papers in International Refereed Journals:

1. **Arun Kumar** and Maneesh Jaiswal **(2016)**. Design and *in-vitro* investigation of nanocomposite hydrogel based *in-situ* spray dressing for chronic wounds and synthesis of silver nanoparticles using green chemistry. *Journal of Applied Polymer Science.* 133, 43260. (**IF-1.76**).
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1. **Arun Kumar** and Maneesh Jaiswal. "A Nanotechnological Approach to Improve Oral Antidiabetic Efficacy of Phytochemicals Extracted from *Tinospora Cordifolia*: *In-Vivo* and *Ex-Vivo* Studies" **Oral presentation** at 'International Conference on Life Sciences, Informatics, Food and Environment (IC LIFE 2014)' in *Jaypee Institute of Information Technology (JIIT), Noida, Uttar Pradesh, INDIA* from 29-30, August 2014.
2. **Arun Kumar**. "Gastro-retentive drug delivery system for Indinavir sulfate: An approach for sustained release with Zero-Order Kinetics" **Poster presentation** at 'International conference on Pharmaceutical sciences' *Division of Pharmaceutical sciences, Shri Guru Ram Rai Institute of Technology*, *Dehradun, Uttarakhand, INDIA* from 14-15, February 2014.